SHERWIN

The Output of Indican as Influenced by Water Drinking & Fasting

Chemistry

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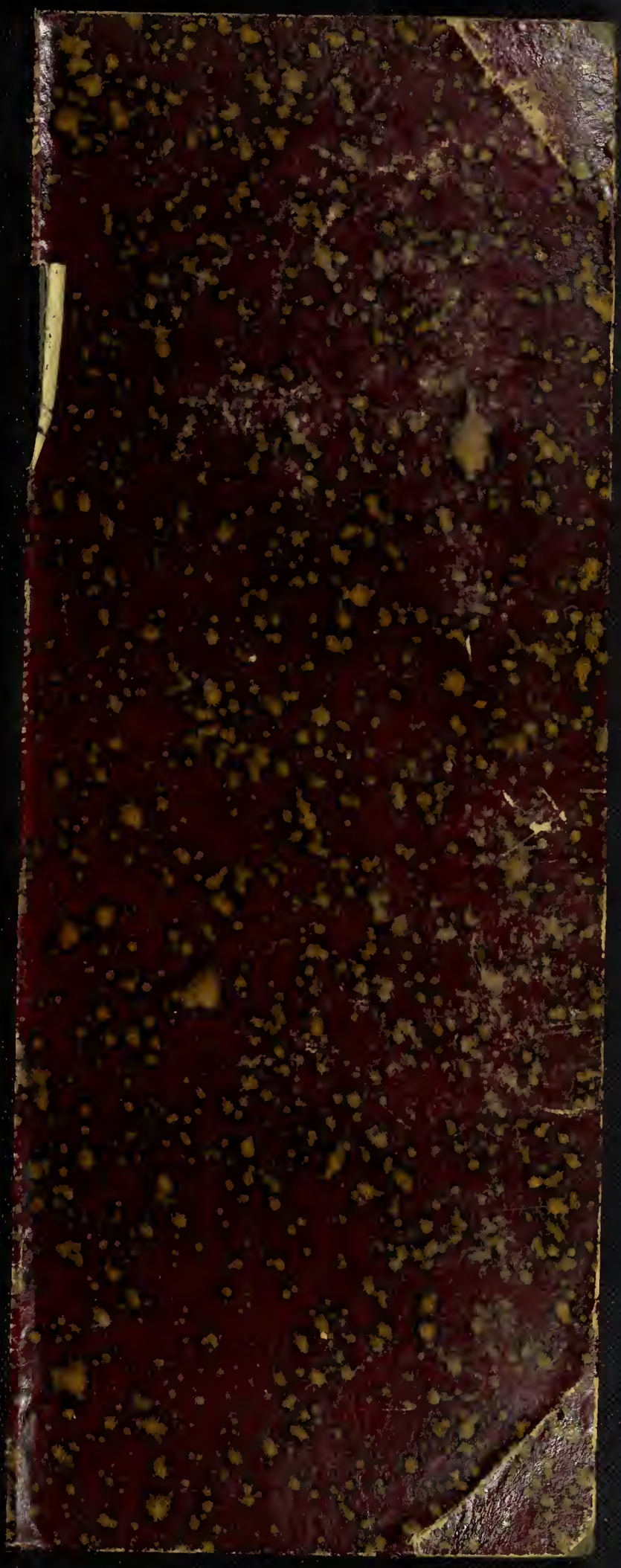

THE OUTPUT OF INDICAN AS INFLUENCED BY WATER DRINKING AND FASTING

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Carl Paxson Sherwin

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TABLE OF CONTENTS.

Part I.

THE OUTPUT OF INDICAN AS INFLUENCED BY WATER DRINKING.

Introduction.

After searching the literature it is seen that an erroneous idea prevails in regard to the drinking of water with meals. This idea, while deeply rooted in popular sentiment, is also fostered by the medical profession of today. This is well summed up in a statement made by Carrington(1) :- "We can lay down the definite and certain rule that it (water) should never be drunk at meals, and preferably not for at least one hour after the meal has been eaten. The effect of drinking water while eating is, first, to artificially moisten the food, thus hindering the normal and healthful flow of saliva and the other digestive juices; secondly, to dilute the various juices to an abnormal extent; and thirdly, to wash the food elements through the stomach and into the intestines before they have had time to become thoroughly liquefied and digested. The effects of this upon the welfare of the whole organism can only be described as direful".

From a scientific standpoint this view is entirely unfounded and lacks the first essentials of experimental proof. On the contrary, we have convincing proof from recent experiments that water-drinking during meals and shortly after same leads to no harmful results and in many cases benefits are derived. Foster and Lambert(2), Heidenhain(3), and Pavlov and Khizhin(4) have reported interesting and important findings. The former experimenters(2) have shown very clearly that the entrance of water into the stomach does not produce a dilute gastric juice of lower acidity but rather

that the entrance of this fluid acts as a distinct stimulation to the gastric secretion and that the juice, although secreted in larger volume than previous to the entrance of the water, nevertheless shows a higher concentration of acid than that juice which is secreted under ordinary conditions. "The copious water-drinking with meals" does not mean the washing down of large amounts of food, but the drinking of water after the food has been thoroughly masticated and swallowed. Fowler and Hawk(5) have shown that copious water drinking (1000 c.c.) with meals decreases the quantity of fecal bacteria per day and increases very decidedly the excretion of urinary nitrogen. Mattill and Hawk(6) after carefully investigating this influence on the utilization of fats, proteins, and carbohydrates conclude that no harmful results follow and show that many benefits result. Hattrem and Hawk(7) moreover believe that intestinal putrefaction is decreased under these conditions, when measured by the excretion of urinary indican.

Description.

In the experiment described in this thesis the subject was placed on a normal, constant diet, and by means of a preliminary period, was brought to a condition of approximate nitrogen equilibrium. At that point 1000 c.c. of water was added to each meal and continued thus through a period of five days. Immediately following this came a final period of eight days, during which the original normal diet was again maintained and the after effects of copious water ingestion observed.

The urine was collected in twenty-four-hour samples. The foods, with the exception of the milk, were analyzed before the

experiment began, after first preparing a satisfactory sample of each of the foods to be used. The milk was analyzed at frequent intervals during the experiment.

The subject of the experiment was a man 22 years of age, weighing at the commencement of the experiment 71.69 kilograms.

The daily schedule for the preliminary period and final periods of the experiment was as follows:

6:30 A. M. Arose, defecated, weighed.
7:30 A. M. Breakfasted (100 c.c. of water with meal).
10:00 A. M. 200 c.c. water taken.
12:30 P. M. Noon meal (100 c.c. water with meal).
3:30 P. M. 200 c.c. water taken.
6:15 P. M. Dinner (100 c.c. water with meal).
8:30 P. M. 200 c.c. water taken.

On the days of increased water-drinking, 1000 c.c. of water was added to each of the three meals. This additional water was to be drunk throughout the progress of the meal, especial care being taken to masticate the food properly.

The articles of diet were butter, peanut butter, whole milk, soda crackers, corn flakes and sugar; 25 grams of butter, 20 grams of peanut butter, 400 grams of whole milk, 100 grams of soda crackers, 25 grams of corn flakes, and 12 grams of sugar constituting the menu for one meal. Each meal of the day was absolutely identical with the others, thus giving an absolute uniformity of diet throughout the experiment. The daily diet contained 14.654 grams of nitrogen.

The water used in the experiment was from the regular University supply. This water analyzed as follows(8):

Analysis of Water from University of Illinois Water Supply.

Ions	Parts per million	Hypothetical combinations	Parts per million	Grains per U.S. gallon.
Potassium,	2.6	Potassium nitrate,	1.1	0.06
Sodium,	29.0	Potassium chloride,	2.9	0.17
Ammonium,	2.3	Sodium chloride,	3.5	0.20
Magnesium,	34.9	Sodium sulphate,	3.6	0.21
Calcium,	70.1	Sodium carbonate,	60.5	3.52
Iron,	1.0	Ammonium carbonate,	6.1	0.36
Aluminium,	1.3	Magnesium carbonate,	121.2	7.07
Nitrate,	.7	Calcium carbonate,	175.2	10.22
Chlorine,	3.5	Iron carbonate,	2.1	0.12
Sulphate,	2.3	Alumina,	2.5	0.15
Silica,	18.9	Silica,	18.9	1.10
		Total------	397.6	23.18

Before using this water it was softened by adding to 30 liters of water, 5 liters of saturated lime water *. After settling the water was filtered and used. The alkalinity of this water was 70 to phenolphthalein and 180 to menthylorange. Its hardness determined by soap solution was 92 parts per million.

* We are indebted to Mr. L. I. Birdsall of the Illinois State Water Survey for the preparation and testing of this water.

The experimental day extended from 7 a.m. to 7 p.m. If possible the subject defecated each morning before breakfast and then immediately weighed himself without clothes. This schedule was followed each day throughout the experiment. The uniformity in the process of defecation is of great importance, especially when the urine is to be examined for indican, as the retention of feces or irregularity of defecation would cause an increased absorption of indol and a corresponding increase of indican in the urine.

Experimental Data and Discussion.

For the quantitative determination of indican in the urine, it is first necessary to oxidize the indican to indigo, and then extract this indigo from the acidified urine by means of some organic solvent. After evaporation the indigo is again dissolved in concentrated sulphuric acid and titrated against a standard solution of potassium permanganate. The end point in the operation is told when the solution takes on a pale straw color. A great many methods have been suggested for the quantitative determination of indican in the human urine, but none should be considered as really "quantitative".

Ellinger's(9) method as modified by Maillard(10) seems to give the most consistent results on fresh human urine. Fifty c.c. of urine is placed in a small beaker and if neutral or alkaline in reaction is made faintly acid with acetic acid. Five c.c. of basic lead acetate(11) is then added to precipitate the inorganic matter and pigment, after which, the solution is mixed <u>well</u> and filtered. Forty c.c. of the clear filtrate is transferred to a separatory funnel, an equal volume of Obermayer's reagent (from 2 - 3 gm.

ferric chloride per liter concentrated hydrochloric acid) is added, and the indigo thus formed extracted with chloroform. This extraction with chloroform should be repeated until the last chloroform extraction is entirely colorless. After carefully comparing the method of Ellinger with that of Maillard(10), we decided to adopt the modification of the latter in washing the chloroform extraction with dilute sodium hydroxide (1 gm. of Sodium hydroxide in 1 liter of distilled water). This is accomplished by placing in a large separatory funnel the entire chloroform extraction with an equal volume of 1/10 per cent sol. of Sodium hydroxide and shaking for several minutes. The extraction is then removed and washed several times with the distilled water, as in the method given above, then separated from the water, placed in an Erlenmeyer flask and evaporated to dryness on a water bath. After the dry residue has been heated for five minutes on the water bath, 10 c.c. of concentrated sulphuric acid is added to each sample and after thoroughly shaking, allowed to remain for ten more minutes on the water bath. Each sample is then diluted with 100 c.c. of distilled water, when it should take on a blue color depending on the concentration of the indigo in solution. As soon as the blue solution has been cooled to room temperature, it is titrated with a very dilute solution of potassium permanganate. The end point is indicated by the dissipation of all the blue color from the solution and the formation of a pale yellow color (12).

In making up the permanganate for titration, an approximate 0.3 per cent solution was first prepared. This stock solution was diluted with forty volumes of water, as suggested by Wang(13). The

dilute solution was then standardized and employed in the titration* 1 c.c. being equal to 0.137 mg. indigo or 0.263 mg. indican.

Maillard's modification of Ellinger's method has been severely criticized by Ellinger(14) on account of its inefficiency, but in all cases we secured a clearer blue solution by following Maillard's instructions. Ellinger's original method calls for several washings of the steam dried residue with hot water and it seems to us that in many cases this leads to an unnecessary loss of indican.

Bouma(15) boiled the urine with hydrochloric acid containing isatin, thereby changing all the indoxyl of the urine into indigo red and titrated this red solution against potassium permanganate. According to our experience this procedure is not satisfactory.

Folin's(16) method is practically the same as Ellinger's except the final titration by means of potassium permanganate is omitted. He extracts the indigo with 5 c.c. chloroform and then determines the amount colorimetrically by comparison with Fehling's solution which he gives an arbitrary value of 100.

Imabuchi(17) conducts his method in much the same manner as Ellinger, except that he uses a few cubic centimeters of a 10 per cent solution of copper sulphate with 40 c.c. of concentrated hydrochloric acid to oxidize the indican to indigo blue, whereas Ellinger uses Obermayer's reagent. By an exhaustive series of experiments he shows, that with copper sulphate it was possible to obtain more indigo, and that it was not necessary to extract immediately with

* We are indebted to Prof. F. P. Underhill of Yale University for the pure indigo used in this titration.

chloroform as was the case when Obermayer's reagent was used. His data shows that the urine may stand as long as ten minutes after the addition of copper sulphate solution before extracting with chloroform and still there will be no loss of indigo by super oxidation, as is true in the case of Obermayer's reagent. He also states that from 1 c.c. to 2 c.c. is usually enough of the copper sulphate to oxidize 50 c.c. of urine but that an excess of the reagent does very little harm.

In the literature we find few references to the quantitative determination of indican in preserved urines. Imabuchi(17) shows that this may be accomplished either by Obermayer's reagent or the copper sulphate solution in cases where either human urines or dog urines have been preserved with chloroform. Hattrem and Hawk(18) were unable to secure accurate titration with potassium permanganate on urines preserved with thymol.

Before taking up this work we were very much interested in Maillard's modification of Ellinger's method, and, after satisfying ourselves that it led to more accurate results with fresh urine, we tried it on a number of old human urines preserved with thymol. The results obtained were identical with those reported by Hattrem and Hawk(18). Our samples when removed from the water bath and diluted with water were either red or dark brown in color instead of blue. In cases where there was a very small amount of indican in the urine this error seemed less prevalent. One sample of urine with almost a negligible amount of indican turned red when the Obermayer's reagent was used, and this red color prevailed in the chloroform solution after extraction but when washed with the 1/10 per cent solution of sodium hydroxide the color changed from light red to pale blue.

This sample was carried through the regular method employed for fresh urines and when titrated with potassium permanganate gave a very clear end point. We next tried thymol-preserved urines which were known to contain much larger amounts of indican and washed the chloroform extracts with a more concentrated sodium hydroxide. In nearly all cases we were able to secure clear blue solutions for titration and the end point after titration was usually a water-clear solution. The concentration of sodium hydroxide best suited for the washing of the chloroform extract proved to be a five per cent solution. This method was thoroughly tested in the following manner:-

Four liters of mixed human urine was collected in April, and six duplicate samples were analyzed for indican, after thoroughly shaking. The samples showed (Table I) that each 50 c.c. of urine contained an average of 1.1576 mg. of indican. Immediately after these six samples were taken from the mixed urine it was preserved with 3 grams of finely powdered thymol, and was shaken for several minutes each time before sampling. This urine was analyzed each week until the following June and then remained in cold storage until the following September, when the analysis was again resumed.

Table I.

Analysis of the Fresh Urines April 21, 1911.

Duplicate samples by number.	1	2	3	4	5	6	Average.
Amount of indican per each 50 c.c. of urine.	1.1592	1.1500	1.1580	1.1590	1.1598	1.1600	1.1576

Table II shows results obtained from the analysis of the thymol-preserved urine on the different dates indicated in the table.

Table II.

Date of analysis.	Number of sample.	No. c.c. of Potassium permanganate used.	Mg. of Indican per 50 c.c. of urine.	Average value of Indican in mg. per 50 c.c. of urine of two duplicates.
Apr. 28, 1911,	1	3.25	1.1765	
Apr. 28, 1911,	2	3.30	1.1846	1.1802
May 5, 1911,	3	3.20	1.1572	
May 5, 1911,	4	3.25	1.1765	1.1667
May 12, 1911,	5	3.20	1.1572	
May 12, 1911,	6	3.20	1.1572	1.1572
May 18, 1911,	7	3.20	1.1572	
May 18, 1911,	8	3.25	1.1765	1.1667
May 26, 1911,	9	3.18	1.1512	
May 26, 1911,	10	3.20	1.1572	1.1542
June 5, 1911,	11	3.15	1.1403	
June 5, 1911,	12	3.20	1.1572	1.1486

Table III shows the results obtained from the analysis of the same mixed urine, beginning October 5, 1911, four months after the last analysis shown in Table II.

Table III.

Date of analysis.	Number of sample.	No. c.c. of Potassium permanganate used.	Mg. of Indican per 50 c.c. of urine.	Average value of Indican in mg. per 50 c.c. of urine of two duplicates.
Oct. 5, 1911,	13	3.15	1.1403	
Oct. 5, 1911,	14	3.15	1.1403	1.1403
Oct. 15, 1911,	15	3.15	1.1403	
Oct. 15, 1911,	16	3.18	1.1512	1.1460
Oct. 30, 1911,	17	3.15	1.1403	
Oct. 30, 1911,	18	3.20	1.1572	1.1485
Nov. 15, 1911,	19	3.20	1.1572	
Nov. 15, 1911,	20	3.18	1.1512	1.1542
Dec. 3, 1911,	21	3.20	1.1572	
Dec. 3, 1911,	22	3.20	1.1572	1.1572
Dec. 26, 1911,	23	3.18	1.1512	
Dec. 26, 1911,	24	3.20	1.1572	1.1542

The indican value for 50 c.c. of fresh urine at the time of collection shows 1.1576 mg. as an average of six duplicate samples (Table I), while in Table III the average of two duplicate samples shows an indican value of 1.1542 mg. for 50 c.c. of the same urine after it had been preserved with thymol for more than eight months.

In the analysis of these samples the regular Ellinger method was followed until the chloroform extract had been secured,

then this chloroform solution of indigo (red) was placed in a large separatory funnel and shaken for several minutes with an equal volume of a five per cent solution of sodium hydroxide. After standing for a few minutes thesodium hydroxide solution forms a very distinct upper layer and is in all cases colored from a light yellow to a pink. If this pink solution is removed and titrated against the solution of potassium permanganate used for the indigo, it will in no wise change color, even after excessive amounts of potassium permanganate have been added to it. The chloroform extract of the indigo was next removed and washed from six to ten times with distilled water as described above. In the case of the preserved urines, however, the washing must be continued for a somewhat longer time in order to remove the last traces of the sodium hydroxide.

The urines analyzed in the water-drinking experiment had been standing in cold storage for about two years, and each sample was preserved with thymol, In the analysis of these samples, except during the water period, 50 c.c. urine was taken, clarified with 5 c.c. of basic lead acetate, and then 40 c.c. of the clear filtrate was used for the determination. During the water period when the urine volume was much larger 150 c.c. of the urine was taken and evaporated to 50 c.c.

Table IV.

Day.	Urine volume expressed in cubic centimeters.	Potassium permanganate used in titrating 40 c.c. of the clarified sample.	Total number of c.c. of Potassium permanganate used to titrate the entire urine volume.	Indican output per day expressed in mg.
		Preliminary Period (3 days).		
1	830	5.90	135.308	35.6
2	920	6.70	168.544	44.1
3	880	4.50	107.888	28.4
Average	877	5.70	137.246	36.0
		Water Period.		
4	3440	3.50	112.832	29.7
5	3840	3.60	125.836	33.1
6	3670	2.90	96.888	25.4
7	3610	3.05	99.997	26.3
8	4020	3.85	104.279	27.4
Average	3716	3.18	107.966	28.3
		Final Period.		
9	1590	6.65	290.971	76.5
10	885	9.83	237.622	62.5
Average	1237	8.24	264.296	69.5

It may be seen from Table IV that the average amount of indican contained in the urine during the "preliminary period" was 36.0 mg. per day, while during the "water period" this average falls to 28.3 mg. per day, thus indicating that water drinking with meals decreases intestinal putrefaction. It has been shown by

previous experimenters (18) that copious water drinking with meals leads to better absorption of protein material by the intestine and corresponding decrease of intestinal putrefaction as measured by the excretion of urinary indican and fecal bacteria.

What apparently took place furing the water period, as far as the digestive organs were concerned was that the entrance of large amounts of water into the stomach stimulated the flow of the gastric juice, thus facilitating the digestion of the protein constituents of the diet. The strongly acid chyme passing into the intestine caused a pronounced stimulation of the pancreatic secretion. The products of gastric digestion were consequently more rapidly and thoroughly digested. The coursing of the large volume of water through the intestine walls stimulated the absorption process and the intestine was thoroughly flushed, thus ridding itself of putrefaction materials.

The most striking feature of the experiment is the manner in which the indican value of the urine suddenly increases from an average of 28.3 mg. per day during the water period to an average of 69.5 mg. during the final period. On the last day of the water period the excretion of 27.4 mg. is representative of that period while the day following we find more than two and one-half times as much in the urine excreted. On the second day of the final period the indican value decreases more than 20 per cent of that of the previous day. In comparing these results with those obtained by Hattrem and Hawk also Sherwin and Hawk we find that in all previous cases reported the urinary indican value becomes much larger immediately after the water period and then gradually decreases until the

average is quite the same as that of the preliminary period. This is more easily understood when we learn (5) that the output of feces during the water period was 74 grams per day but during the final period this rose to 98 grams.

Conclusion.

The daily drinking of three liters of water with meals, for a period of five days, by a man twenty-two years of age, brought into a condition of nitrogen equilibrium through the ingestion of a uniform diet, has shown the following results:-

1- The drinking of large volumes of water with meals decreases intestinal putrefaction.

2- When Ellinger's method or Maillard's modification of Ellinger's method is employed, the urines must be fresh or preserved with chloroform.

3- Urines preserved with thymol may be used for quantitative indican determinations, after standing eight months in cold storage, but in this case the chloroform extract of indigo red must be thoroughly shaken with a five per cent solution of sodium hydroxide and then washed several times with distilled water.

4- The decreased intestinal putrefaction brought about through the ingestion of large amounts of water is probably due to accelerated absorption of the products of protein digestion and the passage of large amounts of strongly acid chyme into the intestine thus inhibiting the growth and activity of indol-forming organisms.

Part II.

THE OUTPUT OF INDICAN AS INFLUENCED BY FASTING.

Introduction.

The literature of fasting is as contradictory as it is interesting. The subjects of most fasts have been lower animals and in the majority of these cases adult dogs have been used. The average life of the animals under these circumstances ranges from thirty to fifty-five days. Falck(19) reports a "normal" fast, however, in which the subject (dog) lived sixty-five days. Kumagawa and Miura(20) report a fast of ninety-eight days in which the animal was subjected to the influence of phlorhizin and for this reason it should not be considered as a normal fast.

The fasting tests in which human beings have served as subjects range in length from two to fifty days. The most complete data furnished in short fasts have been furnished by Benedict(21). Of the longer fasts those on Beaute(22), Tosca(23), Schenck(24), Succi(25), Cetti(26), Breithaupt(27), "E" and "H"(28), Tanner(29), Merlatti(30), and a very recent test by Benedict(31) are the most important. The longest of these are the thirty-day fast of Succi, the fifty-five-day fast of Merlatti, and the very recent fast conducted by Benedict.

Description.

The object of the experiment was to determine the effect of fasting on the urinary indican excretion.

The subject of the experiment was an adult Scotch collie

dog, "oscar", weighing 26.33 kg. at the opening of the fast. At the start of the investigation it was intended to fast the animal to the premortal rise in nitrogen excretion and then to bring him back to normal condition by means of careful feeding and subsequently fast him again. In short it was proposed to make a study of repeated fasting.

The diet used in the preliminary period was as follows:

	grams
Meat	400
Cracker dust	100
Lard	45
Bone ash	12
Water	700

The above diet contained 15.769 grams of nitrogen. Approximate nitrogen equilibrium was secured in eight days. The urine was collected in twenty-four-hour samples.

Several other dogs were subjected to fasts at the beginning of Oscar's fast but it was soon noticed that none of them possessed the same vitality shown by this dog. On the forty-eighth day, when the last of these associated dogs had reached the pre-mortal rise of nitrogen excretion, Oscar was able to jump in and out of his cage which required a leap of about three feet. He continued to jump into his cage until the fifty-eighth day and to jump out of it until the one hundred and first-day of the fast. When the one hundred and seventeenth-day was reached it was decided to terminate the fast. The dog now weighed 9.76 kg. as against 26.33 kg. at the opening of

the fast, a loss of 63 per cent in body weight. It was then June 2 and the dog had been fasting continuously since February 6.

During the pre-fasting interval the dog was given 700 c.c. of water per day. He also received this same amount of water daily during the fast except for an interval of four days, beginning on the fifty-ninth day. During this four-day interval the daily water ingestion was increased to 2100 c.c.

The analytical work on the urine for indican content was not undertaken until several months after the urine was collected, and, like the samples described in Part I, they were placed in glass stoppered bottles carefully labeled and placed in cold storage after being preserved with thymol. The method used for the determination of indican in this case was the same as that mentioned before for thymol preserved urines.

It will be seen from Table V that in many cases we have analyzed a composite sample of urine covering several days. During the four-day copious water interval the urine of each day was analyzed separately. On the sixty-first day of the fast 100 c.c. of the urine was taken, evaporated to 50 c.c., and the concentrated solution analyzed. Again on the sixty-second and sixty-third days 150 c.c. of each day's sample was taken and evaporated to 50 c.c. before analysis. This concentration was made necessary because the urine volume had more than doubled on these three days mentioned.

In Table V will be found the indican values for the "Normal Period" previous to the fast, as well as the entire one hundred and seventeen-day fast.

Table V.

Days.	Average daily urine volume in cubic centimeters.	Potassium permanganate solution used in titrating 40 c.c. of clarified sample.	Total potassium permanganate solution used for 24 hr. average urine volume.	Indican output per day in mg.
		Normal Period (6 days). (700 c.c. water per day).		
5-7	914	3.40	84.82	22.3
9-11	810	4.20	92.91	24.4
Average	862	3.80	88.86	23.4
		Fasting Period (117 days). (700 c.c. water per day).		
1-4	554	6.70	101.38	26.6
5-8	493	3.30	46.01	12.1
30-33	517	5.05	71.35	18.7
36-37	544	2.90	43.10	11.3
54-55	493	2.10	28.24	7.4
56-57	505	1.95	26.11	6.9
58-59	475	2.00	25.93	6.8
Average	511	3.43	48.87	12.8
		Water Ingestion 2100 c.c. per day.		
60	1385	1.25	47.64	12.4
61	2390	1.45	47.56	12.4
62	1685	3.75	57.96	15.2
63	1840	2.20	35.36	9.3
Average	1825	2.16	47.13	12.3

Table V (continued).

Water Ingestion 700 c.c. per day.

64	820	8.50	190.24	50.0
65	640	7.05	120.96	31.8
66	545	5.25	77.94	20.5
67	625	6.40	109.71	28.9
80-82	492	5.15	72.70	19.1
92-95	561	3.20	49.03	12.9
104-107	539	3.78	55.51	14.6
115	514	5.20	72.98	19.2
116	550	4.85	66.60	17.5
117	596	7.00	103.83	27.3
Average	588	5.64	91.95	24.2

The excretion of the total etherial sulphate output was formerly taken as the index of intestinal putrefaction, but the idea now prevails among investigators that the course of intestinal putrefaction is better estimated by following the excretion of urinary indican. The relationship existing between these two factors has already been pointed out by Hattrem and Hawk(7), also Sherwin and Hawk(32), Müller(33) determined the indican in Cetti's urine but failed to find any after the third day of the fast. This is a very surprising finding as both the above named experimenters (7 and 32) found that indican persisted in the urine throughout the fast. Since we are to consider the indican excretion an index of putrefaction, it would be expected that as the fast progressed the indican value would gradually decrease, but the entire absence of

putrefaction after the third day is especially surprising, as juices and secretions containing protein material are continually being poured into the intestine even during inanition.

It may be seen from Table V that the average indican excretion per day during the pre-fasting interval was 23.4 mg. As the fast progressed this amount gradually decreased under the same conditions of water ingestion and on the fifty-ninth day this had decreased to approximately one-fourth that excreted during the pre-fasting interval, 6.8 mg.

During the "Water Ingestion Period" when the water was increased to three times the normal vol me, 2100 c.c. per day, the indican average rose to 12.4 mg. per day which is an increase of almost 100 per cent as compared to the day preceding this period. Immediately after this period when the water ingestion was again reduced to 700 c.c. per day the indican excretion rose to 50. mg. per day which was more than double that of the pre-fasting interval. This is the same result as obtained by Hattrem and Hawk, and Sherwin and Hawk and is also shown in Part I of this thesis when a man on a uniform diet was subjected to copious water-drinking. It might seem strange that after a fast of more than two months there could be protein material enough in the intestine to cause such an excessive output of indican. Various juices and secretions, however, continue to be poured into the intestine during fasting and the protein constituents of the unabsorbed portion (34) of these fluids would form a medium for the development and activity of the varied types of intestinal bacteria, among them the indol-formers (35). This would be true especially after the subject had drunk large volumes of

water, as it has been proven quite conclusively that the ingestion of large amounts of water causes an increased out-pouring of both gastric (4) and pancreatic (36) secretions.

With the low water ingestion, the indican content of the urine again declined until the one hundred and fourth-day of the fast when the amount reached 14.6 mg. per day and continued to rise from that point until the end of the fast when it reached a value of 27.3 mg. During this period of increased excretion the dog had begun to show signs of weakness, and the feces by this time had become principally mats of hair showing that very little, if any, protein material was left unabsorbed. Perhaps this increased indican output was one of the indexes of bodily breakdown, for as Maillard(10) states, excessive amounts of indican usually indicate a strained physical or mental condition of the subject.

Conclusion.

The following conclusions may be drawn from the tabulated data:

1- During the fast the excretion of urinary indican persisted until the end of the fast, probably due to the putrefaction of unabsorbed protein material from the digestive juices.

2- The high water ingestion caused the secretion of a much larger volume of digestive juices into the intestine. It is entirely possible that the absorptive function was less active than usual due to the protracted character of the fast, thus leaving more of the protein material of these juices in the intestine

available for the uses of the putrefactive organisms. In other words, the ingestion of large quantities of water by a normally nourished animal may decrease putrefaction, whereas such high water ingestion might not be able to cause such a decrease in an animal after a protracted fast.

Part III.

THE OUTPUT OF INDICAN AS INFLUENCED BY REPEATED FASTING.

Introduction.

Many cases of repeated fasting have been reported: Albitsky (37) conducted a fast upon two dogs to determine the loss of body weight and the effect of water ingestion at certain periods; Tuvim (38) subjected two dogs to three repeated fasts in order to study the effect of water ingestion on the respiration during a repeated fast; Schulz(39), Mangold(40), Stübel(41), and Hempel(42), fasted several dogs repeatedly until the premortal rise in nitrogen excretion to study the effect of a succeeding low protein and subsequently low carbohydrate diet; Manassein(43), Kagan(44), Ugrumoff(45), Seeland(46), Richet(47), Ranke(48), Schöndorf(49), Leffmann(50), and several others have reported repeated fasts on such subjects as pigeons, rabbits, cats, dogs and rats; but in none of these cases do they mention the effect of repeated fasting on the indican content of the urine. Some of the later articles on longer repeated fasts are those of Howe and Hawk(51), also Howe, Mattill, and Hawk(52). In the former case the experimenters studied the effect of repeated fasting on the nitrogen partition and physiological resistance, while in the latter case the effect of copious water ingestion was studied.

In Part II were discussed the data from an initial fast of one hundred and seventeen days. In this case of repeated fasting the subject was the same Scotch collie dog, "Oscar". After completing his first fast on June 2, he was carefully fed and in a few

weeks was completely restored to his former physical condition. During the summer the dog was kept on a Kansas farm under close observation and before his second fast, the following autumn, had regained his lost body weight and was in good physical condition. Before beginning the second fast he was again brought into nitrogen equilibrium. The diet during this pre-fasting period being the same as that used before the initial fast.

Experimental.

The urines from this fast were not analyzed until several months after the fast, but at the time of collection were placed in glass stoppered bottles preserved with thymol and deposited in cold storage. During this fast there was no period of copious water ingestion and the daily excretions of urine were quite uniform. It was therefore not deemed necessary to take larger amounts of urine and evaporate to the standard sample of fifty c.c. Thus each day's ingestion of water during this one hundred and four day fast was 700 c.c. and each day a sample of 50 c.c. of urine was taken and the indican determined quantitatively as described in Part I of this thesis. On many occasions a sample of mixed urine was taken covering from three to four days. This fact is indicated in the tabulated data.

Table VI.

Days	Average daily urine volume in c.c.	Potassium permanganate solution used in titrating 40 c.c.	Total potassium permanganate solution necessary to titrate the entire day's urine volume.	Indican output per day expressed in mg.
Normal Pre-fasting Period (4 days). 700 c.c. water ingested.				
1-4	504	3.26	45.2	11.9
Fasting Period (104 days). 700 c.c. water ingested per day.				
1-4	596	4.10	66.8	17.6
5-8	448	3.20	39.2	10.3
26-29	595	2.45	39.7	10.4
42-45	509	2.60	36.1	9.5
54-57	619	1.10	18.7	4.9
66-69	563	0.00	0.0	0.0
78-81	603	0.00	0.0	0.0
90-93	613	0.00	0.0	0.0
102-105	438	0.00	0.0	0.0

The average output of indican during the pre-fasting period was 11.9 mg. per day, but in contrast to this we find that the average daily output during the first four days of the fast was 17.6 mg., a rise of fifty per cent over the pre-fasting period. This increase in the output of indican is probably due to the retention of the unabsorbed protein material in the intestine. During

the next period of four days we found the indican value much lower, in fact it decreased 40 per cent of the first four days of the fast. This average continued until the end of the twenty-ninth day. During the next fifteen days of the fast the value decreased to approximately half the output for each of the first four days. The next sample analyzed was that period extending from the fifty-fourth to and including the fifty-seventh day. This sample gave the remarkably low average of 4.9 mg. It was expected that the succeeding samples would give a strong test for indican and a value much higher than that of the last period, however, the most careful work and repeated analysis failed to show more than the merest trace in one sample and that was entirely too small to measure quantitatively. Beginning with the sixty-sixth day and from thence to the end of the fast the indican value of the urine must be considered as zero.

This shows beyond doubt that from the sixty-sixth day, or approximately during the second half of the fast, there was practically no intestinal putrefaction. This may be due to the fact that the animal organism had been so influenced by the repeated fasting as to be able to fully absorb and utilize all the protein material excreted into the intestine(51).

Discussion of the Two Fasts.

Data have been presented from two fasts upon a dog, which had previously never been subjected to an experiment of this kind. The first fast continued for a period of one hundred and seventeen days. This fast was followed by an intermediate feeding period of

four months, during which time the animal was restored to his original condition. Following this came a second fast, which continued for one hundred and four days.

Some of the important factors which affect the length of or intensity of a fast are the race or breed, the age, the physical condition and the activity of the subject, also the character of the pre-fasting diet and the water ingestion during the fast.

These two fasts are comparable in points of preliminary feeding and the water ingestion except on the fifty-ninth day of the first fast (117 days) the water ingestion was increased from 700 c.c. to 2100 c.c. for a period of four days.

During the feeding period previous to the first fast we find a daily average excretion of 23.4 mg. of indican, while during the corresponding period of the second fast this value amounted to only 11.9. Again by comparing the first few days of the fast it is seen that on the first four days of the first fast the average output was 26.6, but in the second fast this same period shows an average of only 17.6 mg. just a little more than half that amount. This relationship holds true in comparing any period of the first fast with a like period of the second, i.e., in the former case the indican value is approximately twice the latter. After the sixty-sixth day, when the indican had entirely disappeared from the urine of the second fast, there still remained in the urine of the first fast an average of 20 mg. until the end of the fast. In fact, the indican output increased instead of decreased during the last twenty-five days of the initial fast.

These data show that during the initial fast the intestinal

putrefaction was greater during any specified interval than it was during a corresponding period of the second fast. It would also appear that in repeated fasting there is a tendency on the part of the animal body toward a conservation of energy and possible food material in the form of digestive secretions.

Conclusions.

1- As a result of this experiment it has been shown that there is less indican excreted in repeated fasting than at any like period during an initial fast.

2- During the first fast the output of indican steadily increases during the last quarter of the fast, but during the second fast the output steadily decreases and disappears during the second half.

3- The animal organism seems to have the power of conserving its energies after undergoing a primary fast as demonstrated by reduced intestinal putrefaction during repeated fasts.

4- In this second fast the reduction of intestinal putrefaction may have been caused by the power of the organism to absorb all possible food material from the intestine.

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* These references were obtained from the English Translation of Pashutin's book, prepared under the direction of Francis G. Benedict of the Carnegie Institute.